CHARACTERIZATION OF THE CAPSID AND CYLINDRICAL INCLUSION PROTEINS OF THREE STRAINS OF TURNIP MOSAIC VIRUS

By

John Gordon McDonald

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Chairman: Dr. E. Hiebert
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Three strains of turnip mosaic virus (TuMV) were compared on the basis of some of the properties of their capsid and cylindrical inclusion proteins. Two of the strains (TuMV-T and TuMV-R) were type I isolates (those TuMV isolates that can infect all _Brassica_ species), while the third strain (TuMV-D) was a type II isolate (those TuMV isolates that only infect a number of _Brassica_ species). Using SDS as the dissociating agent, the capsid protein of TuMV-D was serologically distinct from the capsid proteins of TuMV-T and TuMV-R, but no serological differences were noted between the respective cylindrical inclusion proteins. The cylindrical inclusions induced by TuMV-R were morphologically distinct from those induced by TuMV-D and TuMV-T, when viewed by thin sectioning _in situ_ and by negative staining of purified preparations. While TuMV-D and TuMV-T induced the formation of laminated aggregates that

were long and flat, those induced by TuMV-R were short and curved. No strain differences were noted for the molecular weights of either the capsid or the cylindrical inclusion protein subunits. The fast forms of the capsid protein subunits had a molecular weight of approximately 24,900 d, and the slow forms had a molecular weight of approximately 33,700 d. The cylindrical inclusion protein subunits had a molecular weight of approximately 71,000 d.

INTRODUCTION

Cylindrical inclusions are invariably present in the cytoplasm of tissue infected with potato virus Y (PVY)-group viruses (Edwardson, 1966; 1974), and are often in intimate contact with their causal viruses. Consistent differences in morphology (Edwardson *et al.*, 1968; Purcifull *et al.*, 1970; Edwardson *et al.*, 1972), serological specificity (Hiebert *et al.*, 1971; Purcifull *et al.*, 1973), and subunit molecular weight (Hiebert and McDonald, 1973) between cylindrical inclusions induced by different viruses suggests that these structures are coded for by the viral genome.

In most instances, the study of virus gene products has been limited to the capsid protein, as this is the gene product that appears to be present in the greatest abundance, and is most readily purified. However, in the case of viruses in the PVY group, additional knowledge of the virus genome may be obtained from the characterization of the virus-induced inclusion.

When comparing PVY-group viruses on the basis of the properties of the virus capsid, and of the virus-induced cylindrical inclusion, it is necessary to understand the variability in these properties that can exist between

strains of the same virus. Knowledge of this variability may also help in explaining the relationship between the virus and the cylindrical inclusion it induces.

Strains of PVY-group viruses have been compared on the basis of host range, serology, and inclusion morphology. Partial purification of cylindrical inclusions permits the physical and serological properties of these inclusions to be used as additional criteria.

In this study the criteria used to compare strains of a PVY-group virus include the molecular weight of the capsid and cylindrical inclusion subunits, the serological properties of the virus and cylindrical inclusion, and the morphology of the cylindrical inclusion.

LITERATURE REVIEW

Turnip mosaic virus (TuMV) is a member of the PVY group (Brandes and Bercks, 1965; Harrison *et al.*, 1971; Edwardson, 1974). As such, it is transmitted by aphids (Kennedy *et al.*, 1962), has the shape of a long flexuous rod with a particle length of about 720 nm (Tomlinson and Walkey, 1967), is serologically related to PVY (Shepard *et al.*, 1974), and induces the formation of cylindrical inclusions (Edwardson and Purcifull, 1970).

The host range of TuMV is wide and includes species occurring in 20 dicotyledonous families (Tomlinson, 1970). However, strains have been noted that vary in both host range and symptom severity. Although considerable minor variations exist, most isolates of TuMV may be divided into those that can infect all *Brassica* species, and those that can only infect a number of them (species that fail to be infected include *B. oleracea* L. varieties). For the purposes of this discussion, the former group will be referred to as type I isolates, while the latter group will be referred to as type II isolates.

Type I isolates have been obtained from such species as *B. rapa* L. (Tompkins, 1938), *B. oleracea* L. var. *capitata* L. (Walker *et al.*, 1945), *B. oleracea* L. var.

botrytis L. (Weathers et al., 1972), B. napo-brassica Mill. (Berkeley and Weintraub, 1952), Armoracia rusticana Gaertn. (Pound, 1948), and Limonium perezii Mill. (Niblett et al., 1969). Type II isolates have been obtained from such species as Matthiola incana R. Br. (Tompkins, 1939; Pontis, 1973), B. nigra L. (Sylvester, 1953), Alliaria officinalis Andrz. (Stefanac and Milicic, 1965), Hesperis matronalis L. (Evans, 1972), and Sisymbrium irio L. (Feldman and Gracia, 1972).

Attempts to differentiate isolates of TuMV on bases other than host range have been limited. Using liquid precipitin tests, different isolates of TuMV have been shown, serologically, to be closely related (Larson et al., 1950; Niblett et al., 1969; Feldman and Gracia, 1972), but degrees of relatedness have not been determined. Lack of work in this area has been partly due to the problems that are involved in working with members of the PVY group. Due to the propensity of virus particles in this group to aggregate, difficulties have been experienced in obtaining sufficient quantities of purified virus (Damirdagh and Shepherd, 1970). Another problem, in the past, has been the inability of these viruses to diffuse through an agar gel, thus preventing their use in immunodiffusion tests. Immunodiffusion tests are superior to liquid precipitin tests in many respects: they are simpler to perform, give reliable results when using crude plant extracts, and can indicate partial identity between two antigens by spur

formation (Crowle, 1973). In recent years, however, techniques have been developed for the degradation of the capsid protein of flexuous rod-shaped viruses so that the degradation products may be detected in Ouchterlony double-diffusion tests (Purcifull and Shepherd, 1964).

Various chemical agents have been found that will degrade the capsid proteins of PVY-group viruses, but among the most effective is the detergent, sodium dodecyl sulfate (SDS)(Gooding and Bing, 1970), and the alkalis, ethanolamine (Purcifull and Gooding, 1970), and pyrrolidine (Shepard et al., 1974). Another detergent that has been found to be effective in certain instances (e.g. with celery mosaic virus, CeMV) is sodium dibutylnaphthalenesulfonate (Leonil SA)(Shepard and Grogan, 1967; Sutabutra and Campbell, 1971). Phosphotungstic acid, a chemical that is often used as a negative stain in electron microscopy, has also been shown to be effective at degrading at least one PVY-group virus (Hiebert et al., 1971). The action of most, if not all, of these degrading agents appears to dissociate the viral capsid into protein subunits that are either in monomer or simple aggregate form (Shalla and Shepard, 1970). The antigenic properties of the dissociated protein appear to be quite distinct from those of the mature virion (Shepard and Shalla, 1970; van Regenmortel and Lelarge, 1973).

Degradation of PVY-group viruses may also be achieved by ultrasonic treatment. Tomlinson and Walkey (1967) sonicated TuMV and produced particle fragments that could

be used in Ouchterlony double-diffusion tests. Similar results were obtained with potato virus X (PVX), also a long flexuous rod, but in a different virus group (Brandes and Bercks, 1965). Based on studies with PVX they concluded that the antigenic properties of sonicated and nonsonicated virus were identical.

The serological relationship between strains of a number of PVY-group viruses has been investigated. Snazelle et al. (1971), using a liquid precipitin test, showed differences between a number of strains of maize dwarf mosaic virus, while Sutabutra and Campbell (1971), using the Ouchterlony double-diffusion test in the presence of Leonil SA, showed differences between a number of strains of CeMV. However, serological differences are not always detected between strains of PVY-group viruses. Paguio and Kuhn (1973), using a liquid precipitin test, found that a number of strains of peanut mottle virus were identical. Using Ouchterlony double-diffusion tests, Bond and Pirone (1971) found that a number of strains of sugarcane mosaic virus were identical when ethanolamine was used as the degrading agent, and Gooding and Tolin (1973) found that a number of strains of PVY were identical when SDS was used as the degrading agent.

Other criteria, such as physical and chemical properties, may also be useful in differentiating virus strains. Hill and Shepherd (1972a; 1972b) investigated some of these properties with an isolate of TuMV from

Raphanus sativus L. (host range stated was inadequate for categorizing it as either a type I or type II isolate). The molecular weight of the RNA was estimated at about 3.5×10^6 daltons (d) from its mobility during polyacrylamide gel electrophoresis, while the molecular weight of the capsid protein subunit was estimated to be about 26,000 d from both its mobility during polyacrylamide gel electrophoresis in the presence of SDS, and from amino acid analysis. The virion was found to contain 5 % RNA. The molecular weight of the capsid protein of another isolate of TuMV was investigated by Hiebert and McDonald (1973). Working with a type I isolate from B. rapa (Purcifull, 1968), they found that polyacrylamide electrophoresis in the presence of SDS revealed two protein species with molecular weights of 27,000 d (fast form) and 36,000 d (slow form). The ratio of these two species depended on the history of the virus preparation. In freshly purified preparations the slow form was predominant, while the reverse was true in older preparations.

Similar electrophoretic heterogeneity has been observed for a number of PVY-group viruses (Hiebert and McDonald, 1973; Huttinga and Mosch, 1974). Huttinga and Mosch (1974) attributed this transformation, of slow to fast form, to the action of contaminating proteolytic enzymes, while Hiebert and McDonald (1973) presented evidence that the two forms were charge isomers, a situation analagous to one that has been observed for PVX (Koenig, 1972).

The ultrastructure of cylindrical inclusions induced by PVY-group viruses has been described in detail by Edwardson _et al_. (1968). Thin sectioning studies of the cylindrical inclusions induced by a type I isolate of TuMV (Edwardson and Purcifull, 1970), and by an isolate of unstated origin (Hayashi _et al_., 1965), have demonstrated the presence _in situ_ of pinwheel, tube, and laminated aggregate structures. In similar studies of the inclusions induced by other type I isolates, as well as by TuMV isolates of unstated origin, only tubes and laminated aggregates were observed (Kamei _et al_., 1969a; Russo _et al_., 1972). However, it has been noted that pinwheel structures are not invariably present in tissues infected by PVY-group viruses, but may be observed more readily in newly invaded cells than in cells that have been diseased for a long period of time (Edwardson, personal communication).

In negatively stained tissue extracts, the cylindrical inclusions induced by a type I isolate of TuMV (Edwardson and Purcifull, 1970) appeared as either long narrow rectangular bodies (corresponding to either the curved plates at the central portion of the cylindrical inclusion or the tube structures), or as wide triangular or irregularly shaped bodies (corresponding to the laminated aggregate structures).

The morphology, _in situ_, of cylindrical inclusions induced by strains of PVY (Montenegro _et al_., 1968), bean yellow mosaic virus (Kamei _et al_., 1969b; Weintraub and

Ragetli, 1966; Edwardson et al., 1972), and tobacco etch virus (Matsui and Yamaguchi, 1964; Rubio-Huertos and Hidalgo, 1964) has been compared and no significant strain differences have been observed. This apparent uniformity in inclusion morphology, within strains of the same virus, has led workers to use inclusion morphology as a criterion in virus diagnosis (Purcifull et al., 1970).

Cylindrical inclusions have been partially characterized in situ by the use of certain cytochemical techniques. They have been shown to be digested by proteolytic enzymes (Shepard, 1968; Weintraub and Ragetli, 1968), suggesting that they are largely composed of protein, and have been shown to be antigenically unrelated to the causal virus by use of immuno-ferritin labelling (Shepard and Shalla, 1969).

Partial purification of cylindrical inclusions has permitted their further characterization (Hiebert et al., 1971): spectrophotometric analysis confirmed their protein nature; and they were shown to be serologically distinct from the causal virus when antisera to the virus and inclusion were tested in Ouchterlony double-diffusion tests in the presence of SDS. This serological test has also been used to show that the cylindrical inclusions induced by five PVY-group viruses, including a type I isolate of TuMV, were antigenically distinct, although in some cases related (Purcifull et al., 1973). When the protein subunits of the inclusions induced by these five viruses were analyzed by polyacrylamide gel electrophoresis in the presence of SDS,

they were found to have molecular weights in the range of 67,000-70,300 d (Hiebert and McDonald, 1973). The subunit of the cylindrical inclusion induced by the isolate of TuMV had a molecular weight of 70,300 d.

MATERIALS AND METHODS

Source and Culture of the Virus Strains

The following strains of TuMV were obtained: a turnip (_B. rapa_) isolate from Florida (Purcifull, 1968), and designated TuMV-T; a rutabaga (_B. napo-brassica_) isolate from Ontario, Canada (Evans, 1972), and designated TuMV-R; and a Dames violet (_H. matronalis_) isolate from Ontario (Evans, 1972), and designated TuMV-D. The latter two isolates were kindly supplied by I.R. Evans, Department of Environmental Biology, University of Guelph, Ontario.

The virus cultures were maintained in _B. perviridis_ Bailey by mechanical transmission, using cold deionized water as the medium for sap extraction. To reduce the possibility of contamination with other viruses, the cultures used were derived from single local lesion infections on _Nicotiana tabacum_ L. Samsun NN (Purcifull, 1968).

Additional hosts of TuMV that were used in this study included a _Nicotiana_ hybrid (Christie, 1969), and _Zinnia elegans_ Jacq. All plants were grown in air-conditioned greenhouses in cages that excluded aphids.

Virus and Cylindrical Inclusion Purification

Purification of TuMV-T, TuMV-R, and TuMV-D, and the

cylindrical inclusions they induced, designated TuMVI-T, TuMVI-R, and TuMVI-D, was performed essentially as described previously (Hiebert and McDonald, 1973). Urea was not used in virus purification, and the only modification in virus purification was that deionized water was used as the resuspension medium. All three viruses and their respective cylindrical inclusions were purified from B. perviridis, while TuMV-T and TuMVI-T were also purified from the Nicotiana hybrid. Inclusion yield was determined as described previously (Hiebert et al., 1971; Hiebert and McDonald, 1973). Virus yield was estimated by assuming the extinction coefficient of TuMV to be 2.4/cm for a 1 % solution at 261 nm (Purcifull, 1966).

Purified virus preparations were stored at 4 C in the presence of approximately 0.05 % sodium azide, while inclusion preparations were stored at -20 C by either freezing directly or after lyophilization.

Virus and Cylindrical Inclusion Serology

Preparation of Antisera

Antigens were emulsified 1:1 with Freund's incomplete adjuvant and injected intramuscularly into rabbits. Periodically, blood was collected from the ear and clotted by incubation at 37 C. The clot was removed by centrifugation at 300 g for 15 min, and the serum was stored at -20 C by either freezing directly, or after lyophilization. Serum that was in current use was kept at 4 C in the

presence of approximately 0.05 % sodium azide.

Virus antisera to each strain were prepared by immunizing two rabbits with virus that had been freshly purified from B. perviridis and had been subjected to equilibrium centrifugation in cesium chloride. In addition, a single rabbit was immunized with similar preparations of TuMV-T that had been purified from the Nicotiana hybrid. The rabbits received two intramuscular injections, each containing 1-10 mg of virus, and administered 2-3 weeks apart.

Antisera were prepared to inclusions that had been purified from B. perviridis. Two rabbits were immunized with TuMVI-T, while single rabbits were immunized with TuMVI-R and TuMVI-D. The rabbits received two intramuscular injections, each containing 5-10 optical density units at 280 nm, and administered 6-17 weeks apart.

Testing of Antisera

Ouchterlony double-diffusion was used to test the various antisera. In general, the detergent method of Gooding and Bing (1970) was used. The gels consisted of 0.8 % Noble agar, 0.5 % SDS, and 1 % sodium azide. Purified antigens were degraded in 1 % SDS, while antigens in plant extracts were prepared by grinding leaf tissue in water and 3 % SDS (1 g tissue:1 ml water:1 ml 3 % SDS) (Purcifull et al., 1973). Treated antigens were sometimes stored at -20 C by either freezing directly or after lyophilization.

Virus antigens were also degraded by treatment with the following: 3 % pyrrolidine; 3 % ethanolamine; 4 % phosphotungstate; and 1 % Leonil SA. Treatments were made at room temperature, and treated preparations were incubated for approximately 1 hr prior to testing. Antigens degraded in all but the latter degrading agent were tested in gels composed of 0.8 % Noble agar and 0.1 % sodium azide in 0.05 M borate, pH 8. Antigens degraded in Leonil SA were tested in gels composed of 0.8 % Noble agar, 0.1 % sodium azide and 1 % Leonil SA. Antigens in plant extracts (1 g tissue:2 ml water) were lyophilized prior to the addition of the degrading agents.

In general, purified antigens were used to test the specificity of the antisera, while antigens in plant extracts were used to examine the serological relationships between the strains. In some tests some of the antisera (prepared to antigens purified from _B_. _perviridis_) reacted positively with plant extracts from noninoculated controls, suggesting the presence in these antisera of antibodies to normal plant components. These antibodies were removed by absorption with extracts purified from noninoculated _B_. _perviridis_. Extracts were purified by high speed centrifugation by the method of Purcifull _et al_. (1973). Absorption was performed either overnight at 4 C, or for 2 hr at 37 C. The resulting precipitate was removed by centrifugation at 9000 _g_ for 15 min. In some cases the absorbed antisera were lyophilized and readjusted to their original volume.

Wells (7 or 10 mm in diameter) were cut in the agar gels with an adjustable gel-cutter (Grafar Corp.). Peripheral wells were spaced 3-4 mm from the center well. After addition of the reactants, the plates were incubated several days in a moist chamber at 24 C, and results were observed periodically and recorded photographically.

Electron Microscopy of Cylindrical Inclusions

All material was examined with a Philips 200 electron microscope, operating with an accelerating voltage of 60 KV. Photographs were made on 35 mm film.

Negative Staining

Negatively stained cylindrical inclusions, which were examined both in crude leaf tissue extracts and in purified preparations, were mounted on Formvar carbon-coated 70 X 300 mesh copper grids. Tissue extracts were made in 1 % ammonium molybdate (adjusted to neutrality and containing 0.05 % bovine serum albumin) by the leaf chopping method (Purcifull *et al.*, 1970). Purified preparations were mounted on grids and stained with either 1 % ammonium molybdate or 2 % uranyl acetate.

Thin Sectioning

The morphology of the cylindrical inclusions induced by the three strains of TuMV was examined *in situ* by the use of thin sectioning. Infections of each strain were established in young *B. perviridis* plants of identical age. After 16 days leaf samples were taken from inoculated and

systemically infected portions of these plants. Control samples were taken from noninoculated plants. Samples, 1-2 mm^2, were fixed for 2 hr at 4 C in Karnovsky's formaldehyde-glutaraldehyde fixative in the presence of 0.1 M cacodylate buffer, pH 7.2 (Karnovsky, 1965). They were washed for 4 hr at 4 C in the buffer, and postfixed in 2 % osmium tetroxide at room temperature. After rinsing for 10 min in two changes of buffer at 4 C, they were progressively dehydrated in a series of increasing concentrations of ethanol to 100 %. At 70 % ethanol they were stained overnight in 2 % uranyl acetate at 4 C. They were subsequently transferred to 100 % acetone at room temperature, and embedded in Mollenhauer's No. 2 plastic containing Epon 812 and Araldite 502 (Mollenhauer, 1964).

Thin sections were cut with a diamond knife on a Sorvall model MT-1 ultramicrotome. They were mounted on Formvar carbon-coated 100 mesh copper grids, stained for 10 min in 2 % aqueous uranyl acetate, and rinsed in deionized water. Subsequently they were stained for 2 min in 0.1 % lead citrate (Venable and Coggeshall, 1965), and were rinsed in deionized water.

Polyacrylamide Gel Electrophoresis of Capsid and Cylindrical Inclusion Proteins

Polyacrylamide gel electrophoresis of the capsid and cylindrical inclusion proteins of the three virus strains was performed as described previously (Hiebert and McDonald, 1973).

RESULTS

Virus and Cylindrical Inclusion Serology

In Ouchterlony double-diffusion tests in the presence of SDS (Gooding and Bing, 1970), the various antisera reacted with their respective homologous purified antigens, and had dilution end points between $\frac{1}{2}$ and $\frac{1}{4}$. In addition, TuMV-T, TuMV-D, and TuMV-R reacted with the heterologous virus antisera, and TuMVI-T, TuMVI-D, and TuMVI-R reacted with the heterologous inclusion antisera, but no cross-reactivity was observed between virus and inclusion antisera. Antisera prepared in different rabbits to the same antigen showed similar specificity. None of the antigens reacted with normal serum.

Although heterologous reactions were observed between the virus antigens, TuMV-D appeared to be serologically distinct from TuMV-R and TuMV-T. Figure 1 shows the homologous and heterologous reactions of virus antisera to the three strains in plant extracts of _B. perviridis_. Antisera to TuMV-T (virus immunogen purified from _B. perviridis_ and the _Nicotiana_ hybrid) and TuMV-R show heterologous spur formation with respect to TuMV-D, while the antiserum to TuMV-D shows heterologous spur formation with respect to TuMV-T and TuMV-R.

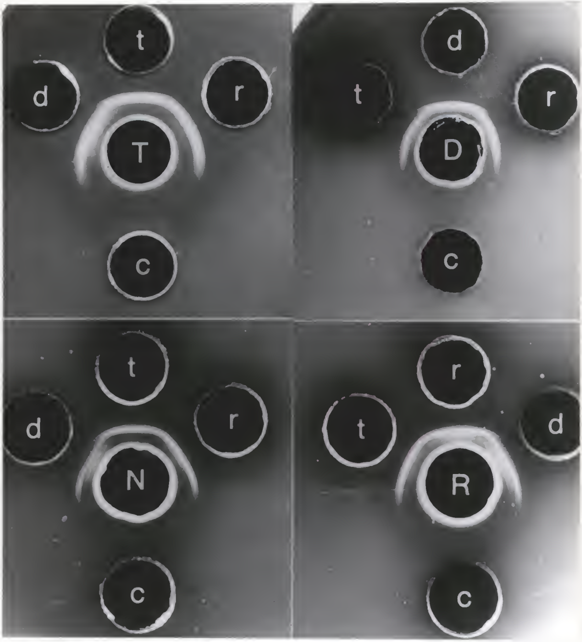


Figure 1.

Homologous and heterologous reactions of TuMV-T, TuMV-D, and TuMV-R to their respective antisera. The center wells contain antisera. Anti-TuMV-T sera, prepared to antigen purified from B. perviridis and the Nicotiana hybrid, are designated T and N, respectively. Anti-TuMV-D and anti-TuMV-R sera are designated D and R, respectively. The peripheral wells contain antigens in SDS-treated extracts from B. perviridis. Antigens from noninoculated plants and plants infected by TuMV-T, TuMV-D, and TuMV-R, are designated c, t, d, and r, respectively.

The inclusion antigens, however, appeared to be serologically identical. Figure 2 shows the homologous and heterologous reactions of inclusion antisera to the three strains in plant extracts from B. perviridis. No spur formation is evident.

The plant host had no apparent effect on the antigenic specificity of the virus and inclusion antigens. Reactions of partial identity between TuMV-D and the other two strains were observed when extracts from infected Z. elegans were used as antigens. Extracts of B. perviridis, Z. elegans, and the Nicotiana hybrid infected by TuMV-T gave reactions of identity when tested with antisera to TuMV-T and TuMVI-T (Fig. 3).

The serological relationship between the virus antigens was also tested using pyrrolidine, ethanolamine, phosphotungstate, and Leonil SA as degrading agents. However, as is shown in Fig. 4, no distinct spur formation was observed between the heterologous antigens.

It was discovered during the course of this investigation that storage at 4 C affected the serological specificity of the virus antigen. Freshly purified virus was serologically identical to the same virus in plant extracts, while purified virus that had been stored for some time showed only partial identity (by spur formation) to freshly purified virus. To maintain the antigenic specificity of freshly purified virus it was dissociated in 1 % SDS and stored at -20 C by either freezing directly or

Figure 2.

Homologous and heterologous reactions of TuMVI-T, TuMVI-D, and TuMVI-R, to the respective antisera, designated IT, ID, and IR, respectively. Antisera are in the center wells. The peripheral wells contain antigens in SDS-treated extracts from B. perviridis. Antigens from non-inoculated plants and plants infected by TuMV-T, TuMV-D, and TuMV-R are designated c, t, d, and r, respectively.

Figure 3.

Homologous reactions of TuMV-T and TuMVI-T in different hosts. The center wells contain antisera. Anti-TuMV-T sera, prepared to antigen purified from B. perviridis and the Nicotiana hybrid, are designated T and N, respectively. Anti-TuMVI-T serum is designated IT. The peripheral wells contain antigens in SDS-treated extracts from the Nicotiana hybrid, B. perviridis and Z. elegans. Extracts from noninoculated plants of the three species are designated h, p, and e, respectively, and extracts from the plants infected by TuMV-T are designated th, tp, and te, respectively.

Figure 4.

Reactions of TuMV-T, TuMV-D, and TuMV-R degraded by pyrrolidine (top left), ethanolamine (top right), phosphotungstate (bottom left), and Leonil SA (bottom right), to anti-TuMV-T serum. The center wells contain anti-TuMV-T serum, designated T. The peripheral wells contain antigens in treated extracts from B. perviridis. Antigens from noninoculated plants and plants infected by TuMV-T, TuMV-D and TuMV-R, are designated c, t, d, and r, respectively.

after lyophilization. Figure 5 shows the serological relationship of a plant extract of TuMV-T from B. perviridis to two purified preparations of TuMV-T: one stored as above to maintain its serological specificity (fresh), and the other stored for 15 months at 4 C (old). The plant extract shows identity to the fresh preparation, but spurs over the old preparation. Similarly, when the fresh and old preparations were placed adjacent to one another, the former spurred over the latter.

Electron Microscopy of Cylindrical Inclusions

Negative Staining

Striated cylindrical inclusion structures were observed in negatively stained extracts of B. perviridis infected by TuMV-T, TuMV-D, and TuMV-R. Extracts of tissue infected by either TuMV-T or TuMV-D contained long narrow rectangular inclusion bodies and wide triangular or irregularly shaped inclusion bodies, while extracts of tissue infected by TuMV-R contained only long narrow rectangular inclusion bodies.

As a number of PVY-group viruses exclusively induce the formation of either tubular or laminated aggregate inclusions (Edwardson, 1974), it was speculated that the presence of both these structures (e.g. in tissue infected by TuMV-T) may result from the presence of a mixture of strains; i.e. one strain might induce the formation of tubular inclusions, while another might induce the

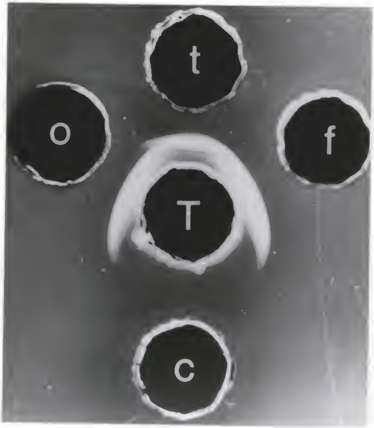


Figure 5.

Effect of storage at 4 C on the serological specificity of purified TuMV-T. The center well contains anti-TuMV-T serum, designated T. The peripheral wells contain the following SDS-treated antigens: purified preparations of TuMV-T, stored 15 months at 4 C, and stored at -20 C immediately after purification, designated o and f, respectively; extracts from noninoculated and TuMV-T-infected B. perviridis, designated c and t, respectively.

formation of laminated aggregate inclusions. In order to separate a possible mixture of strains TuMV-T was inoculated to the local lesion host Chenopodium amoranticolor Coste and Reyn. The negatively stained extracts of 15 lesions were examined, and in each case, both the long narrow rectangular inclusion bodies, and the wide triangular or irregularly shaped inclusion bodies (laminated aggregates) were present. As the laminated aggregate inclusion type was present in each case, these results did not support the possibility that a separable mixture of strains was responsible for the formation of tubular and laminated aggregate inclusions.

Purified cylindrical inclusion preparations of TuMVI-T, TuMVI-D, and TuMVI-R, negatively stained with either uranyl acetate or ammonium molybdate, contained inclusion types similar to those observed in tissue extracts. Figures 6, 7, and 8 show preparations of TuMVI-T, TuMVI-D, and TuMVI-R, respectively, negatively stained with 2 % uranyl acetate. Both the long narrow rectangular inclusion bodies, and the wide triangular or irregularly shaped inclusion bodies are evident in the preparations of TuMVI-T and TuMVI-D, while only the former inclusion type is evident in the preparation of TuMVI-R.

Thin Sectioning

Figures 9, 10, and 11 show cylindrical inclusions in tissue systemically infected by TuMV-T, TuMV-D, and TuMV-R, respectively. As is shown in Figs. 9 and 10, pinwheel, tube, and long flat laminated aggregate structures were

Figure 6.

Electron micrograph of purified TuMVI-T showing long narrow rectangular inclusion bodies (R), and wide triangular or irregularly shaped inclusion bodies (T). Inclusions were negatively stained with 2 % uranyl acetate. Calibration 0.5 μm.

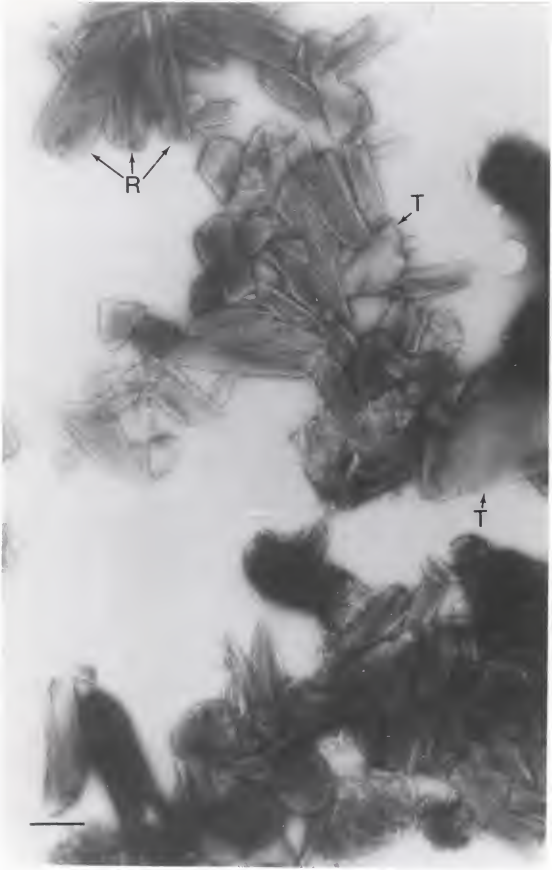

R
T
T

Figure 7.

Electron micrograph of purified TuMVI-D showing long narrow rectangular inclusion bodies (R), and wide triangular or irregularly shaped inclusion bodies (T). Inclusions were negatively stained with 2 % uranyl acetate. Calibration 0.5 μm.

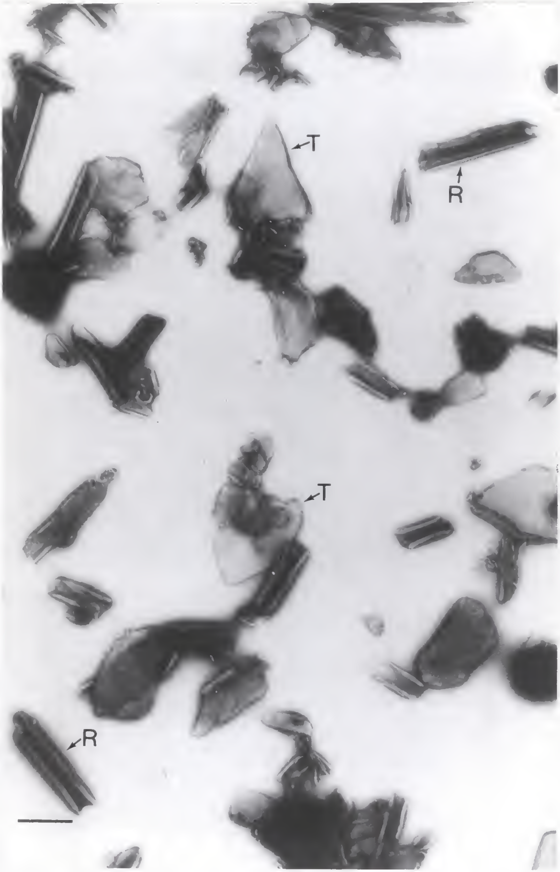

T
R
T
R

Figure 8.

Electron micrograph of purified TuMVI-R showing long narrow rectangular inclusion bodies (R). Inclusions were negatively stained with 2 % uranyl acetate. Calibration 0.5 µm.

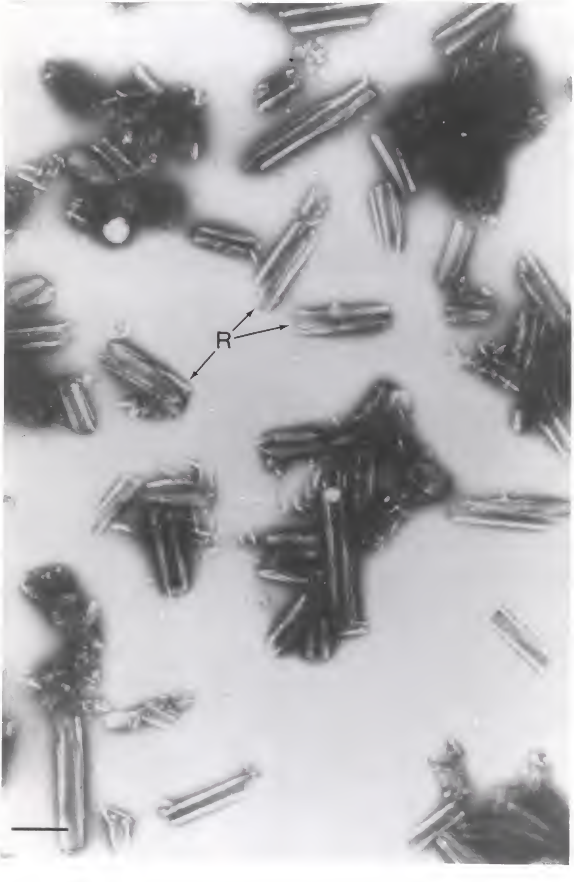

R

Figure 9

Electron micrograph of a thin section of B. perviridis infected by TuMV-T. Cross-sectional views showing pinwheel (PW), tube (T), and laminated aggregate (LA) inclusions. Many of these laminated aggregates show a long and flat morphology. Calibration 0.5 µm.


LA
T
PW

Figure 10.

Electron micrograph of a thin section of _B. perviridis_ infected by TuMV-D. Cross-sectional views showing pinwheel (PW), tube (T), and laminated aggregate (LA) inclusions. Many of these laminated aggregates show a long and flat morphology. Calibration 0.5 μm.

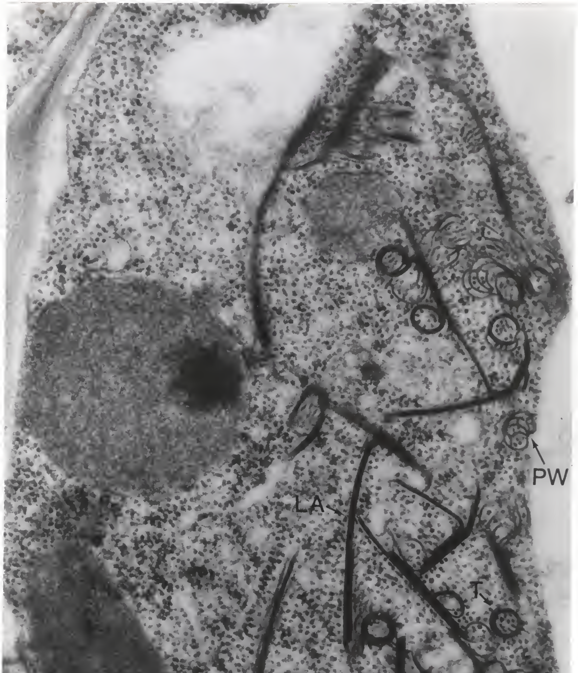
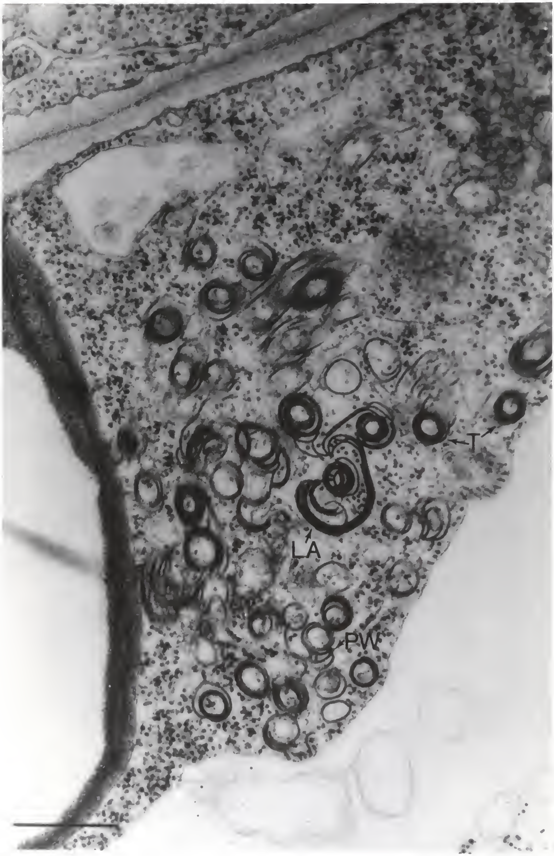

PW
LA
T

Figure 11.

Electron micrograph of a thin section of _B. perviridis_ infected by TuMV-R. Cross-sectional views showing pinwheel (PW), tube (T), and laminated aggregate (LA) inclusions. These laminated aggregates show a short and curved morphology. Calibration 0.5 μm.


T
LA
PW

evident in tissues infected by TuMV-T and TuMV-D. Pin-wheel, tube, and laminated aggregate structures were evident in tissue infected by TuMV-R (Fig. 11), but the laminated aggregates were short and curved, and were, therefore, distinctly different from the long flat structures observed in tissues infected by the other two strains. For each virus strain, the cylindrical inclusions observed in inoculated leaves were morphologically similar to those observed in systemically infected leaves.

Polyacrylamide Gel Electrophoresis of Capsid and Cylindrical Inclusion Proteins

The SDS-dissociated capsid and cylindrical inclusion proteins of the three virus strains were electrophoresed in 6 % polyacrylamide gels. Slow and fast forms of the capsid proteins were observed and the respective forms of the three strains showed similar electrophoretic mobilities. Figure 12 shows a typical gel slab with the slow and fast forms of TuMV-T, TuMV-D, and TuMV-R, as well as tobacco mosaic virus protein (17,500 d), carbonic anhydrase (29,000 d), ovalbumin (43,000 d), glutamate dehydrogenase (53,000 d), and bovine serum albumin (67,000 d) that were used as marker proteins and whose molecular weights are in parentheses. On the basis of two estimates, the molecular weight of the fast forms was estimated to be approximately 24,900 d, and the molecular weight of the slow forms was estimated to be approximately 33,700 d.

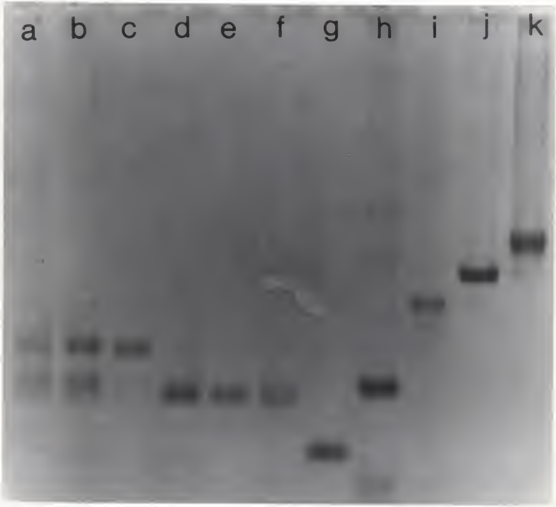
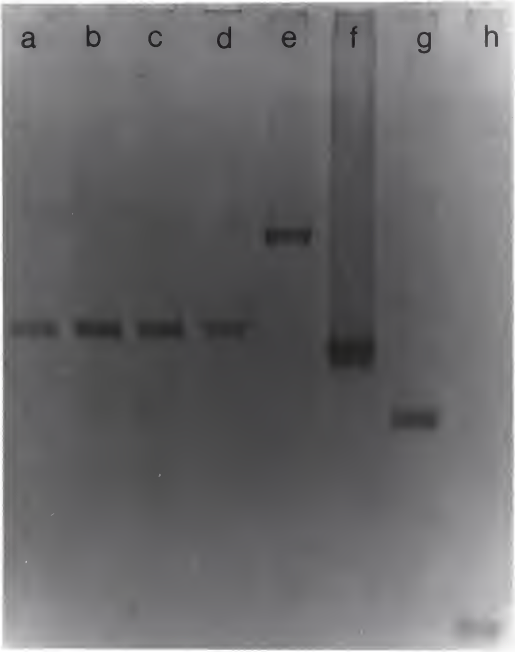


Figure 12.

Electrophoresis of capsid protein subunits and marker proteins in a 6 % polyacrylamide gel containing 0.1 % SDS and sodium phosphate buffer (pH 7.2). Electrophoresis was from top to bottom. Columns from left to right with molecular weights of marker proteins indicated in parentheses: (a) TuMV-R, slow and fast forms; (b) TuMV-T, slow and fast forms; (c) TuMV-D, slow form; (d) TuMV-R, fast form; (e) TuMV-T, fast form; (f) TuMV-D, fast form; (g) tobacco mosaic virus (17,500 d); (h) carbonic anhydrase (29,000 d); (i) ovalbumin (43,000 d); (j) glutamate dehydrogenase (53,000 d); (k) bovine serum albumin (67,000 d).

Figure 13.

Electrophoresis of cylindrical inclusion protein subunits and marker proteins in a 6 % polyacrylamide gel containing 0.1 % SDS and sodium phosphate buffer (pH 7.2). Electrophoresis was from top to bottom. Columns from left to right with molecular weights of marker proteins indicated in parentheses: (a) TuMVI-R, from _B. perviridis_; (b) TuMVI-D, from _B. perviridis_; (c) TuMVI-T, from _B. perviridis_; (d) TuMVI-T, from the _Nicotiana_ hybrid; (e) phosphorylase a (94,000 d); (f) bovine serum albumin (67,000 d); (g) glutamate dehydrogenase (53,000 d); (h) carbonic anhydrase (29,000 d).


a
b
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h

Electrophoresis of TuMVI-T, TuMVI-D, and TuMVI-R protein subunits revealed a single species in each case. The three inclusion proteins purified from B. perviridis, and TuMVI-T purified from the Nicotiana hybrid, were analyzed and all showed similar electrophoretic mobilities. Figure 13 shows a typical gel slab with TuMVI-T, TuMVI-D, and TuMVI-R purified from B. perviridis and TuMVI-T purified from the Nicotiana hybrid, as well as phosphorylase a (94,000 d), bovine serum albumin, glutamate dehydrogenase, and carbonic anhydrase that were used as marker proteins. On the basis of two estimates, the molecular weight of the inclusion proteins was estimated to be approximately 71,000 d.

Table 1 summarizes the results obtained for TuMV-T, TuMV-R, and TuMV-D with respect to host range, virus and cylindrical inclusion serology, and cylindrical inclusion morphology.

Table 1 Summary of results obtained for TuMV-T, TuMV-R, and TuMV-D with respect to host range, virus and cylindrical inclusion serology, and cylindrical inclusion morphology.

TuMV strain	Host of origin	Host range type*	Antisera: Virus	Antisera: Cylindrical inclusion	Cylindrical Inclusion Structure: Negative staining	Cylindrical Inclusion Structure: Thin sectioning
T	Turnip	I	Identical to R and distinct from D	Identical to R and D	Long narrow rectangular, and triangular or irregularly shaped bodies	Pinwheel, tube, and laminated aggregate (long and flat)
R	Rutebaga	I	Identical to T and distinct from D	Identical to T and D	Long narrow rectangular shaped bodies	Pinwheel, tube, and laminated aggregate (short and curved)
D	Dames violet	II	Distinct from R and T	Identical to R and T	Long narrow rectangular, and triangular or irregularly shaped bodies	Pinwheel, tube, and laminated aggregate (long and flat)

* Type I isolates of TuMV include those isolates that can infect all _Brassica_ species, and type II isolates include those that can only infect a number of them (species that fail to be infected include _B. oleracea_ varieties).

DISCUSSION

This study suggests that TuMV-T, TuMV-D, and TuMV-R are distinct strains of TuMV. Host range and serological tests showed that TuMV-D was distinct from TuMV-T and TuMV-R, and study of the morphology of the virus-induced cylindrical inclusions showed that TuMV-R was distinct from TuMV-T and TuMV-D.

Antigenic differences between the dissociated viral capsids were demonstrated by Ouchterlony double-diffusion in the presence of SDS. Preliminary tests using pyrrolidine, ethanolamine, phosphotungstate, and Leonil SA as virus degrading agents failed to show these differences. The reason for this failure is not clear, but it is possible that the antigenic sites produced by dissociation with SDS differ from those produced by the other degrading agents. If this is true it would dispute the claim by Shepard _et al._ (1974) that the antigenic specificities of capsid proteins of PVY-group viruses dissociated by SDS and pyrrolidine are identical.

Although the capsid protein of TuMV-D was found to be antigenically distinct from those of the other two strains, no antigenic differences were detected between the respective dissociated cylindrical inclusion proteins. This

lack of difference between the cylindrical inclusions induced by antigenically distinct virus strains is in contrast to the antigenic differences that have been reported between the cylindrical inclusions induced by different PVY-group viruses (Purcifull *et al*., 1973). However, it appears that closely related virus strains induce the formation of cylindrical inclusions that are also closely related.

Representatives of three plant families, Cruciferae (*B. perviridis*), Compositae (*Z. elegans*), and Solanaceae (the *Nicotiana* hybrid) were used as virus hosts to determine if the host had any effect on the antigenic specificity of the capsid and cylindrical inclusion antigens. No effects were noted, and with respect to the findings with the cylindrical inclusion antigens, these results further support the similar observations of Purcifull *et al*. (1973).

It was found that storage at 4 C resulted in changes in the antigenic specificity of purified virus preparations. Similar losses in antigenic specificity were observed for other PVY-group viruses (unpublished data of the author), so this appears to be a general phenomenon for PVY-group viruses that are stored in this manner. The cause of this loss in antigenic specificity has not been determined, but changes in the conformation of the capsid protein, and the action of proteolytic enzymes are two possible causes. Unknowing use of such altered preparations as either immunogens or test antigens when antisera are tested by

Ouchterlony double-diffusion in the presence of SDS could provide misleading information. For example, when such altered preparations of TuMV-D, TuMV-T, and TuMV-R were tested by Ouchterlony double-diffusion in the presence of SDS no serological differences were detected between them. Whether such changes may be detected when other test systems are used remains to be determined, but in systems where such changes are observed a reevaluation of the data that have been obtained thus far in light of this finding would be desirable.

Significant differences in cylindrical inclusion morphology were observed between the cylindrical inclusions induced by TuMV-R and those induced by TuMV-T and TuMV-D. Whereas the latter two strains induced the formation of long flat laminated aggregates, TuMV-R induced the formation of laminated aggregates that were short and curved. Consistent with this observation _in situ_, the wide triangular or irregularly shaped bodies (interpreted to be the long flat laminated aggregates) were absent from negatively stained preparations of TuMVI-R.

The cylindrical inclusions induced by TuMV-T and TuMV-D were indistinguishable and were similar to those previously described for TuMV-T (Edwardson and Purcifull, 1970; Hiebert and McDonald, 1973), while those induced by TuMV-R were unlike any that have been described for a TuMV isolate. The finding of this unusual cylindrical inclusion morphology emphasizes the caution that should be taken in

using inclusion morphology as a criterion in virus diagnosis.

Examination of negatively stained cylindrical inclusions in individual local lesions did not provide evidence that it was possible to separate populations of TuMV that were capable of inducing exclusively either tubular or laminated aggregate inclusions. It seems likely that the cylindrical inclusions induced by a particular virus are assembled from a single type of subunit. Why some cylindrical inclusion laminae are rolled into tube-shaped scrolls, and others are stacked into flat laminated aggregates, is not known. It is probable that subunit bonding, both inter- and intra-laminae, plays an important part. In the case of the laminated aggregates induced by tobacco etch virus, it has been shown, using freeze-etch electron microscopy, that subunits on adjacent laminae are stacked vertically (McDonald and Hiebert, 1974), suggesting a highly specific subunit bonding.

The cylindrical inclusions induced by TuMV-R are distinct from those induced by TuMV-T and TuMV-D, yet no serological differences between the respective SDS-dissociated subunits were detected. If there are amino acid sequence differences between the subunits of TuMVI-R and the subunits of TuMVI-T and TuMVI-D, they are probably not drastic differences. The possibility still exists, however, that antigenic differences may exist between the respective assembled cylindrical inclusions. It may be

possible to investigate this possibility by cross-absorption in a liquid precipitin system, but this has not been attempted in the present study.

The three strains could not be differentiated on the basis of the mobility of their SDS-dissociated capsid and cylindrical inclusion subunits in polyacrylamide gels. The molecular weight estimates made for the capsid and cylindrical inclusion subunits were within the limits previously reported for TuMV-T (Hiebert and McDonald, 1973). The cylindrical inclusions purified from B. perviridis and the Nicotiana hybrid appeared to have subunits of identical molecular weight, providing further evidence that the host has no influence on the formation of cylindrical inclusions.

The apparent similarities in subunit molecular weight between serologically distinct capsids and morphologically distinct cylindrical inclusions are not surprising. The capsid proteins of some PVY-group viruses have been found to have identical molecular weights (Huttinga and Mosch, 1974; Uyemoto et al., 1972), while others show only slight differences (Hiebert and McDonald, 1973). Slight differences in molecular weight have also been noted between the cylindrical inclusion subunits induced by a number of PVY-group viruses, even though inclusion morphologies varied considerably (Hiebert and McDonald, 1973).

The storage-induced transformation of the capsid protein, from the slow electrophoretic form to the fast form, that has been noted previously for PVY-group viruses

(Hiebert and McDonald, 1973; Huttinga and Mosch, 1974), was also noted in this study. Another storage-induced change in the capsid protein, discussed above, was with respect to its antigenic specificity. As these two changes apparently coincided with one another a preliminary attempt was made to determine if preparations that gave the fast form had lost part of their antigenic specificity. Such preparations were compared to the same virus in crude plant extracts by Ouchterlony double-diffusion in the presence of SDS, and it was indeed found that such preparations had lost part of their antigenic specificity. Similar electrophoretic forms of the capsid protein have been noted for PVX-group viruses (Koenig et al., 1970), but no antigenic differences have been detected between the two forms. Koenig (1972) compared the antigenic specificity of slow and fast forms of PVX and cactus virus X by cross-absorption with whole virus antigen, and Shepard and Secor (1972) excised the SDS-degraded slow and fast forms of PVX from a polyacrylamide gel and compared them by Ouchterlony double-diffusion.

In this study three strains of TuMV were compared on the basis of some of the properties of their capsid and cylindrical inclusion proteins. Some of the properties were shared by two or more strains, but each strain had unique features. Further investigation of the properties of these and other strains of PVY-group viruses should provide further insight into understanding the relationship

between the virus and the cylindrical inclusion it induces.

REFERENCES

Berkeley, G.H., and Weintraub, M. (1952). Turnip Mosaic. Phytopathology 52, 258-260.

Bond, W.P., and Pirone, T.P. (1971). Purification and properties of sugarcane mosaic virus strains. Phytopathol. Z. 71, 56-65.

Brandes, J., and Bercks, R. (1965). Gross morphology and serology as a basis for classification of elongated plant viruses. Advan. Virus Res. 11, 1-24.

Christie, S.R. (1969). Nicotiana hybrid developed as a host for plant viruses. Plant Disease Rep. 53, 939-941.

Crowle, A.J. (1973). Immunodiffusion. 2nd Edit. Academic Press, New York, 545 p.

Damirdagh, I.S., and Shepherd, R.J. (1970). Purification of the tobacco etch and other viruses of the potato Y group. Phytopathology 60, 132-142.

Edwardson, J.R. (1966). Electron microscopy of cytoplasmic inclusions in cells infected with rod-shaped viruses. Amer. J. Bot. 53, 359-364.

Edwardson, J.R. (1974). The potato virus Y group. Florida Agricultural Experiment Station Monograph, in press.

Edwardson, J.R., and Purcifull, D.E. (1970). Turnip mosaic virus-induced inclusions. Phytopathology 60, 85-88.

Edwardson, J.R., Purcifull, D.E., and Christie, R.G. (1968). Structure of cytoplasmic inclusions in plants infected with rod-shaped viruses. Virology 34, 250-263.

Edwardson, J.R., Zettler, F.W., Christie, R.G., and Evans, I.R. (1972). A cytological comparison of inclusions as a basis for distinguishing two filamentous legume viruses. J. Gen. Virol. 15, 113-118.

Evans, I.R. (1972). Occurrence and pathogenicity of turnip mosaic virus isolates (TuMV) collected in southwestern Ontario. Proc. Can. Phytopath. Soc. 39, 30.

Feldman, J.M., and Gracia, O. (1972). Studies of weed plants as sources of viruses. II. Eruca sativa, Rapistrum rugosum and Sisymbrium irio, new natural hosts for turnip mosaic virus. Phytopathol. Z. 73, 115-122.

Gooding, G.V., and Bing, W.W. (1970). Serological identification of potato virus Y and tobacco etch virus using immunodiffusion plates containing sodium dodecyl sulfate. Phytopathology 60, 1293.

Gooding, G.V., and Tolin, S.A. (1973). Strains of potato virus Y affecting flue-cured tobacco in the southeastern United States. Plant Disease Rep. 57, 200-204.

Harrison, B.D., Finch, J.T., Gibbs, A.J., Hollings, M., Shepherd, R.J., Valenta, V., and Wetter, C. (1971). Sixteen groups of plant viruses. Virology 45, 356-363.

Hayashi, T., Matsui, C., and Yamaguchi, A. (1965). Electron microscopy of intracellular turnip mosaic virus. Phytopathology 55, 458-461.

Hiebert, E., and McDonald, J.G. (1973). Characterization of some proteins associated with viruses in the potato Y group. Virology 56, 349-361.

Hiebert, E., Purcifull, D.E., Christie, R.G., and Christie, S.R. (1971). Partial purification of inclusions induced by tobacco etch virus and potato virus Y. Virology 43, 638-646

Hill, J.H., and Shepherd, R.J. (1972a). Biochemical properties of turnip mosaic virus. Virology 47, 807-816.

Hill, J.H., and Shepherd, R.J. (1972b). Molecular weights of plant virus proteins by polyacrylamide gel electrophoresis. Virology 47, 817-822.

Huttinga, H., and Mosch, W.H.M. (1974). Properties of viruses of the potyvirus group. 2. Buoyant density, S value, particle morphology, and molecular weight of the coat protein subunit of bean yellow mosaic virus, pea mosaic virus, lettuce mosaic virus, and potato virus Y^N. Neth. J. Pl. Path. 80, 19-27.

Kamei, T., Goto, T., and Matsui, C. (1969a). Turnip mosaic virus multiplication in leaves infected with cauliflower mosaic virus. Phytopathology 59, 1795-1797.

Kamei, T., Honda, Y., and Matsui, C. (1969b). Intracellular appearance of turnip mosaic and bean yellow mosaic virus particles. Phytopathology 59, 139-144.

Karnovsky, M.J. (1965). A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. J. Cell Biol. 27, 137 A.

Kennedy, J.S., Day, M.F., and Eastop, V.F. (1962). A conspectus of aphids as vectors of plant viruses. Commonwealth Inst. Ent., London. 114 p.

Koenig, R. (1972). Anomalous behavior of the coat proteins of potato virus X and cactus virus X during electrophoresis in dodecyl sulfate-containing polyacrylamide gels. Virology 50, 263-266.

Koenig, R., Stegemann, H., Francksen, H., and Paul, L. (1970). Protein subunits in the potato virus X group. Determination of molecular weights by polyacrylamide electrophoresis. Biochim. Biophys. Acta 207, 184-189.

Larson, R.H., Matthews, R.E.F., and Walker, J.C. (1950). Relationships between certain viruses affecting the genus Brassica. Phytopathology 40, 955-962.

Matsui, C., and Yamaguchi, A. (1964). Electron microscopy of host cells infected with tobacco etch virus. I. Fine structures of leaf cells at later stages of infection. Virology 22, 40-47.

McDonald, J.G., and Hiebert, E. (1974). Ultrastructure of cylindrical inclusions induced by viruses of the potato Y group as visualized by freeze-etching. Virology 58, 200-208.

Mollenhauer, H.H. (1964). Plastic embedding mixtures for use in electron microscopy. Stain Tech. 39, 111-114.

Montenegro, M.J., Kitajima, E.W., de Camargo, I.J.B., and Costa, A.S. (1968). Comparação electronomicroscópica dos tęcidos de plantos infetadas par diferentes estirpes do vírus Y da batata que ocorrem no estado de São Paulo. Bragantia 27, 17-23.

Niblett, C.L., Paulus, A.O., and Semancik, J.S. (1969). A mosaic disease of statice caused by turnip mosaic virus. Phytopathology 59, 1166-1167.

Paguio, O.R., and Kuhn, C.W. (1973). Strains of peanut mottle virus. Phytopathology 63, 976-980.

Pontis, R.E. (1973). Turnip mosaic virus on annual stock in Argentina. Plant Disease Rep. 57, 379-382.

Pound, G.S. (1948). Horseradish mosaic. J. Agr. Res. 77, 97-114.

Purcifull, D.E. (1966). Some properties of tobacco etch virus and its alkaline degradation products. Virology 29, 8-14.

Purcifull, D.E. (1968). Occurrence of turnip mosaic virus in Florida. Plant Disease Rep. 52, 759-760.

Purcifull, D.E., Edwardson, J.R., and Christie, S.R. (1970). A morphological comparison of inclusions induced by tobacco etch and potato Y viruses. Phytopathology 60, 779-782.

Purcifull, D.E., and Gooding, G.V. (1970). Immunodiffusion test for potato Y and tobacco etch viruses. Phytopathology 60, 1036-1039.

Purcifull, D.E., Hiebert, E., and McDonald, J.G. (1973). Immunochemical specificity of cytoplasmic inclusions induced by viruses in the potato Y group. Virology 55, 275-279.

Purcifull, D.E., and Shepherd, R.J. (1964). Preparation of the protein fragments of several rod-shaped plant viruses and their use in agar gel diffusion tests. Phytopathology 54, 1102-1108.

Rubio-Huertos, M., and Hidalgo, F.G. (1964). Ultrathin sections of intranuclear and intracytoplasmic inclusions induced by severe etch virus. Virology 24, 84-90.

Russo, M., Quacquarelli, A., Castellano, M.A., and Martelli, G. (1972). Some ultrastructural features of plants infected with viruses of the potato Y group. Phytopathol. Mediterr. 11, 113-121.

Shalla, T.A., and Shepard, J.F. (1970). An antigenic analysis of potato virus X and of its degraded proteins. II. Evidence for a conformational change associated with the depolymerization of structural protein. Virology 42, 835-847.

Shepard, J.F. (1968). Electron microscopy of subtilisin-treated tobacco etch virus nuclear and cytoplasmic inclusions. Virology 36, 20-29.

Shepard, J.F., and Grogan, R.G. (1967). Serodiagnosis of western celery mosaic virus by double diffusion tests in agar. Phytopathology 57, 1136-1137.

Shepard, J.F., and Secor, G.A. (1972). The effects of enzymatic digestion on the molecular weight and antigenic specificity of potato virus X protein. Phytopathology 62, 1154-1160.

Shepard, J.F., Secor, G.A., and Purcifull, D.E. (1974). Immunochemical cross-reactivity between the dissociated capsid proteins of PVY group plant viruses. Virology 58, 464-475.

Shepard, J.F., and Shalla, T.A. (1969). Tobacco etch virus cylindrical inclusions: antigenically unrelated to the causal virus. Virology 38, 185-188.

Shepard, J.F., and Shalla, T.A. (1970). An antigenic analysis of potato virus X and of its degraded protein. I. Evidence for the degree of antigenic disparity. Virology 42, 825-834.

Snazelle, T.E., Bancroft, J.B., and Ullstrup, A.J. (1971). Purification and serology of maize dwarf mosaic and sugarcane mosaic viruses. Phytopathology 61, 1059-1063.

Stefanac, Z., and Milicic, D. (1965). Zelleinschlüsse des Kohlrübenmosaik-virus. Phytopathol. Z. 52, 349-362.

Sutabutra, T. and Campbell, R.N. (1971). Strains of celery mosaic virus from parsley and poison hemlock in California. Plant Disease Rep. 55, 328-332.

Sylvester, E.S. (1953). Host range and properties of Brassica nigra virus. Phytopathology 43, 541-546.

Tomlinson, J.A. (1970). Turnip mosaic virus. C.M.I./A.A.B. Descriptions of Plant Viruses No. 8.

Tomlinson, J.A., and Walkey, D.G.A. (1967) Effects of ultrasonic treatment of turnip mosaic virus and potato virus X. Virology 32, 267-278.

Tompkins, C.M. (1938) A mosaic disease of turnip. J. Agr. Res. 57, 589-602.

Tompkins, C.M. (1939). Two mosaic diseases of annual stock. J. Agr. Res. 58, 63-77.

Uyemoto, J.K., Providenti, R., and Schroeder, W.T. (1972). Serological relationship and detection of bean common and bean yellow mosaic viruses in agar gel. Ann. Appl. Biol. 71, 135-142.

van Regenmortel, M.H.V., and Lelarge, N. (1973). The antigenic specificity of different states of aggregation of tobacco mosaic virus protein. Virology 52, 89-104.

Venable, J.H., and Coggeshall, R. (1965). A simplified lead citrate stain for use in electron microscopy. J. Cell Biol. 25, 407-408.

Walker, J.C., LeBeau, F.J., and Pound, G.S. (1945). Viruses associated with cabbage mosaic. J. Agr. Res. 70, 379-404.

Weathers, L.G., Gumpf, D.J., and Endo, R.M. (1972). A disease of 'green ball' cauliflower caused by turnip mosaic virus. Plant Disease Rep. 56, 441-442.

Weintraub, M., and Ragetli, H.W.J. (1966). Fine structure of inclusions and organelles in Vicia faba infected with bean yellow mosaic virus. Virology 28, 290-302.

Weintraub, M., and Ragetli, H.W.J. (1968). Intracellular characterization of bean yellow mosaic virus-induced inclusions by differential enzyme digestion. J. Cell Biol. 38, 316-328.

BIOGRAPHICAL SKETCH

John Gordon McDonald was born in London, England on March 26, 1947. He received his secondary school education at Mill Hill School, London. In August of 1964 he emigrated with his parents to Montreal, Canada, and subsequently became a Canadian citizen. He attended Lower Canada College for one year, and in September of 1965 entered Macdonald College of McGill University where he received a Batchelor of Science degree in Agriculture, with a major in plant pathology, in May, 1969. He subsequently entered graduate school at McGill University, and under the supervision of Dr. R.I. Hamilton received a Master of Science degree from the Department of Plant Pathology in October of 1971. His thesis research concerned the distribution and fate of southern bean mosaic virus in the seedcoat of Phaseolus vulgaris. He continued his graduate education at the University of Florida and commenced work towards a Doctor of Philosophy degree, with a major in plant pathology and a minor in botany, in September of 1971.

He is a member of the Canadian and American Phytopathological Societies.

He was married to the former Amanda Jane Robinson on June 13, 1970, and has a one-year-old daughter, Zoe Melissa.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Ernest Hiebert

E. Hiebert, Chairman
Assistant Professor of
Plant Pathology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

H.C. Aldrich

H.C. Aldrich
Associate Professor of
Botany

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

J.R. Edwardson

J.R. Edwardson
Professor of Agronomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

D.E. Purcifull

D.E. Purcifull
Associate Professor of
Plant Pathology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

R.E. Stall

R.E. Stall
Professor of Plant Pathology

This dissertation was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August, 1974

Dean, College of Agriculture

Dean, Graduate School